

MICROBIOLOGY LAB

Module 6: Staining Methods

OUTLINE

PRELAB:

1. Culture Media Preparation to Analysis
2. Preparation of Bacterial Smear
3. Fixation

Heat Fixation

Chemical Fixation

Preparation & Fixation of Smear

4. Dyes

Basic Dyes

Acidic Dyes

5. Staining

LAB INSTRUCTIONS:

6. Introduction

7. Making smears

8. Simple Stain

9. Gram Stain

10. Endospore Stain (Schaeffer-Fulton Method)

11. Acid Fast Stain

POST-LAB

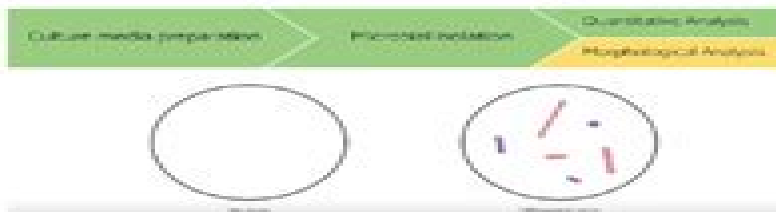
Legend:

Remember

Lecturer

Book

CULTURE MEDIA PREPARATION TO ANALYSIS



PREPARATION OF BACTERIAL SMEAR

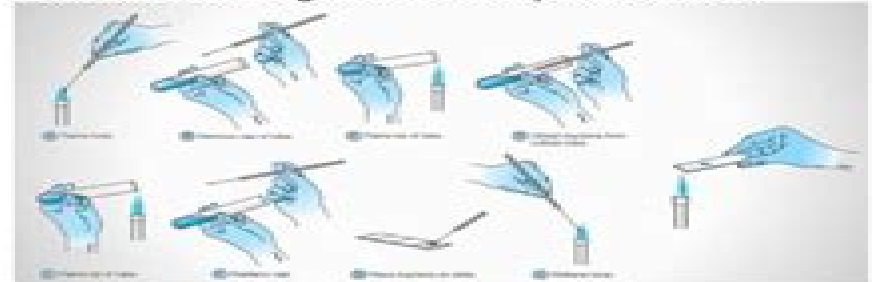
A. Steps

- Flame loop
- Remove cap of tube
- Flame tip of tube
- Obtain bacteria from culture tube
- Flame tip of tube
- Replace cap

- Place bacteria on slide
- Re-flame loop

B. Important steps in the preparation of bacterial smear from broth culture

- If using a broth culture, the liquid is placed directly on the slide.
- If a slant is used, a loopful of water is placed on the slide before adding the small sample of bacteria.



FIXATION

- Is the process by which the internal and external structures of specimens are preserved and fixed in position.
- Inactivates the enzymes that might disrupt cell morphology and toughens cell structures so that they can do not change during staining and observation

A. Heat Fixation

- Firm of cells (smear) is gently heated as a slide is passed through a flame
- Preserves overall morphology but also destroys proteins that may be part of subcellular structures

B. Chemical Fixation

- Chemical Preservatives (e.g., ethanol, acetic acid, mercuric chloride, formaldehyde mixture) penetrate cells and react with cellular components
- Used to protect fine cellular substructure renders protein and lipids to be inactive, insoluble and immobile.

C. Preparation and Fixation of Smear & Heat

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M Walker



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